

Synergizing Mouse and Human Studies to Understand the Heterogeneity of Obesity

Penny Gordon-Larsen,^{1,2} John E French,^{1,3} Naima Moustaid-Moussa,⁴ Venkata S Voruganti,^{1,3} Elizabeth J Mayer-Davis,¹ Christopher A Bizon,⁵ Zhiyong Cheng,⁶ Delisha A Stewart,^{1,3} John W Easterbrook,¹ and Saame Raza Shaikh¹

¹Department of Nutrition, Gillings School of Global Public Health and School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA; ²Carolina Population Center, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA; ³Nutrition Research Institute, University of North Carolina at Chapel Hill, Kannapolis, NC, USA; ⁴Obesity Research Institute and Department of Nutritional Sciences, Texas Tech University, Lubbock, TX, USA; ⁵Renaissance Computing Institute, University of North Carolina at Chapel Hill, NC, USA; and ⁶Food Science and Human Nutrition Department, University of Florida, Gainesville, FL, USA

ABSTRACT

Obesity is routinely considered as a single disease state, which drives a “one-size-fits-all” approach to treatment. We recently convened the first annual University of North Carolina Interdisciplinary Nutrition Sciences Symposium to discuss the heterogeneity of obesity and the need for translational science to advance understanding of this heterogeneity. The symposium aimed to advance scientific rigor in translational studies from animal to human models with the goal of identifying underlying mechanisms and treatments. In this review, we discuss fundamental gaps in knowledge of the heterogeneity of obesity ranging from cellular to population perspectives. We also advocate approaches to overcoming limitations in the field. Examples include the use of contemporary mouse genetic reference population models such as the Collaborative Cross and Diversity Outbred mice that effectively model human genetic diversity and the use of translational models that integrate -omics and computational approaches from pre-clinical to clinical models of obesity. Finally, we suggest best scientific practices to ensure strong rigor that will allow investigators to delineate the sources of heterogeneity in the population with obesity. Collectively, we propose that it is critical to think of obesity as a heterogeneous disease with complex mechanisms and etiologies, requiring unique prevention and treatment strategies tailored to the individual. *Adv Nutr* 2021;12:2023–2034.

Keywords: heterogeneity, obesities, mouse, human, pre-clinical, clinical, prevention, treatment, nutrition, symposium

Introduction

Like cancer decades ago, obesity is considered by many as a single disease state with universal treatment, rather than as a fundamentally heterogeneous process varying in mechanisms and etiologies, each requiring unique prevention and treatment strategies. This “one-size-fits-all” approach has not served patients or communities well; obesity and

its complications continue to rise with significant health-care burden. This article, the result of the first annual University of North Carolina Interdisciplinary Nutrition Sciences Symposium, focuses on a set of factors important to translational science in studies aiming to synergize human and animal models of obesity heterogeneity. In addition, we provide suggestions for best practices to delineate the sources of heterogeneity that will ultimately lead to a better understanding of underlying mechanisms and precision medicine/precision nutrition treatment approaches.

Obesity is Highly Heterogeneous with Many Underlying Sources

Obesity is defined as excess body fat. The most common clinical practice for adults is to use body mass index [BMI; wt (kg)/ht (m)²] to screen and diagnose overweight/obesity to identify cardiometabolic risks (1). However, it is important to recognize that the BMI does not distinguish lean and fat body compartments, as such BMI is problematic on its

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Abbreviations used: BAT, brown adipose tissue; CC, collaborative cross; CCRIL, collaborative cross recombinant inbred lines; DO, diversity outbred; GWAS, genome-wide association studies; IBD, identical by descent; MAF, minor allele frequency; KG, ROBOKOP Knowledge Graph; QTL, quantitative trait loci; SNP, single nucleotide polymorphism; UCP-1, uncoupling protein 1; WAT, white adipose tissue.

TABLE 1 Potential sources of heterogeneity in obesity that should be further integrated between rodent and human studies

Sources of heterogeneity	Description
Sex	<ul style="list-style-type: none"> Sex differences are well established in human and rodent metabolism in the context of obesity. However, more work is needed in integrating sex-specific studies, particularly at the rodent level, with human studies.
Race/ethnic background	<ul style="list-style-type: none"> It is critical to incorporate race/ethnic differences in human studies as they give rise to heterogeneity in the human population. These results can then guide mechanistic experiments at the rodent level.
Age at onset of obesity	<ul style="list-style-type: none"> Rodent studies that compare outcomes with humans need to account for differences in the age of onset of obesity. Furthermore, comparing data within the human population also needs to account for the age of onset and duration of obesity.
Genetic background	<ul style="list-style-type: none"> Host genetics are a well-established source of heterogeneity in the human population. Pre-clinical studies could increasingly incorporate the use of CC and DO mouse models to better understand the role of host genetics in obesity heterogeneity. The next steps will be to integrate these data with human studies.
Tissue/cellular heterogeneity	<ul style="list-style-type: none"> As an example, adipose tissue depots are not uniform within a human or a mouse. Thus, dissecting the role of specific adipose tissue depots on metabolic outcomes is of significance. The abundance and function of differing cell types (e.g., adipocytes, immune cells) within a given tissue can vary considerably. Thus, there is a need to understand how heterogeneity at the tissue and cellular levels gives rise to variation in humans with obesity and rodent models.

CC, collaborative cross; DO, diversity outbred.

own for clinical use at the individual level for estimating body fat and lean mass (2). In addition, at any given BMI, there is heterogeneity in body fat distribution as well as differential association with cardiometabolic disease risk (1). For this reason, recent clinical guidance suggests including measures of waist circumference at given BMI values to improve risk stratification across age, sex, and ethnicity (3). Indeed, The International Diabetes Federation recommends sex- and ethnic-specific waist circumference cut points (e.g., for Asian populations) to allow for the differential risk across populations (4).

The complex disease of obesity results from a range of individual predisposition factors (including genetic, epigenetic, biologic, hormonal, microbial, early life events) as well as a range of environment (geography, nutritional status, contaminant exposures) and lifestyle factors (including built/physical environment, cues/social habits, food cost/availability, taste/smell/palatability) (5, 6). Individual predispositions shape responses to environment and lifestyle factors; derangements in this system lead to obesity. This complex multifactorial etiology makes it challenging to identify the mechanisms and causes of obesity heterogeneity. In addition, there is a paucity of well-characterized, population representative datasets that have information on individual predisposition factors as well as the range of obesity-relevant environment and lifestyle factors. Furthermore, the computational and methodological complexity of analyzing multi-omic and multilevel data in large population datasets cannot be understated.

It is beyond the scope of this review to discuss the intricate details of each source of heterogeneity in the population with obesity (7–9). Herein, we focus on key factors that are critical in synergizing human and animal models of obesity heterogeneity, which are summarized in **Table 1**. One notable factor in the heterogeneity of obesity is sex. At the same BMI, females tend to have more body fat than men. Body composition tends to be sexually dimorphic with central adiposity a strong indicator of cardiometabolic risk. People

with a pear shape tend to carry weight in the hip area, while people with an apple shape tend to have excess fat in the abdominal area, a more cardiometabolically adverse patterning due to metabolically active adipose as energy metabolism and endocrine functions vary with locations of fat deposition (10). There is suggestion that central adiposity may confer higher cardiovascular risk among women than men (11). Understanding such sex differences may shed light on the pathophysiology of adiposity and offer insight for potential interventions aimed at women versus men.

Even within a given sex, there are additional factors that must be considered such as race. For example, gay, lesbian, or gender queer adults may have differential patterns of cardiovascular risk with obesity (12). Further, there are established differences in BMI cut points for Asians given higher cardiovascular risk at lower BMI (4). Similar differences are likely for other race/ethnic groups (13–15) and may result from a combination of biological and structural societal factors such as racism (16). As one example, gluconeogenesis in premenopausal black women is lower than in white women, which has strong implications for diagnosis of pre-diabetes (17). This research gap is increasingly being addressed with a recent requirement, when possible, by the NIH for inclusion of women and populations underrepresented in research involving human subjects and the requirement for addressing sex as a biological variable in research using rodent models.

Besides sex, race, and ethnicity, age at onset of obesity may give rise to obesity heterogeneity. Childhood obesity is associated with increased risk of numerous complications, including but not limited to type 2 diabetes, malignancies, autoimmunity, psychiatric problems, reproductive complications, etc. (18, 19). Of course, more work is needed in this area of research as there is also some discrepancy in the field as many have failed to disentangle duration of obesity with age at onset or have not adequately addressed the complex multifactorial nature of obesity. For instance, one study that focused on adult candidates for bariatric surgery showed that

lower age at onset of obesity predicted higher BMIs; however, these same individuals were less prone to hypertension and type 2 diabetes compared with those with adult-onset obesity (18). Nevertheless, the age at onset, duration of obesity, and other complex factors are critical research gaps to consider in studies of obesity and its complications from the pre-clinical to clinical level. Furthermore, when comparing mouse and human data, the age at onset of obesity may be a factor that is routinely ignored and could be a factor that can improve synergizing mouse and human studies of obesity (20).

Genetics is a major source of heterogeneity in the human population. Most genetic studies of obesity susceptibility have failed to utilize quantitative diet data to interrogate gene variations in obesity that may only be revealed when considering diet. For example, a gene polymorphism that decreases thermogenesis (i.e., rate at which calories are burned) may be of no consequence in people with a low-calorie diet but will influence weight in high calorie consumers. In studies that ignore differential exposures (e.g., pooling responders and non-responders), gene susceptibility is missed. Similarly, few obesity interventions are tailored to individual susceptibility, even to well-established susceptibility factors such as glycemic status. Furthermore, few collect genetic, metabolomic, or microbial data, prohibiting investigation of differential treatment effects and identification and characterization of underlying biologic pathways. We discuss these issues in greater detail below on how to potentially bridge this gap with the integration of newer mouse models for obesity and systems approaches for human research.

While obesity is typically associated with metabolic abnormalities and cardiometabolic diseases, there is individual variation in this risk with differences in patterning of disease risk across obesity, including some individuals with obesity with few cardiometabolic complications. It is well known that obesity perturbs metabolic pathways (21), thereby affecting cardiovascular disease (CVD) risk factors (e.g., cholesterol, blood pressure, and glycemic phenotypes) and their sequelae (22–28) as well as heterogeneity in association with a range of other diseases, from cancers (29) to infections (30). In fact there are many papers classifying people with metabolically healthy obesity, albeit with a large range in definitions and classifications (31). Yet it is important to note that a range of modifiable lifestyle factors, adipose tissue biology, or differential mechanisms (in addition to methodological differences in classification and temporal effects) may underlie differences in metabolic health within the population with obesity (32–35). These differences can point to subtypes of obesity and shed light on mechanisms underlying the heterogeneity of obesity.

We know little about exactly how obesity stresses metabolic pathways during younger adulthood when CVD risk accelerates; the specific biologic mechanisms remain poorly understood (36, 37). Such research gaps reflect several challenges, including a preponderance of studies evaluating lifecycle period *after* CVD is established (38–42). There is a clear need to better understand the evolution of CVD

in the context of unremitting metabolic stress induced by obesity (43, 44) and CVD risk factors (45–48). The “expressed genome”—factors beyond DNA such as epigenomics and metabolomics—offers innovative opportunities to fill this major research gap (49–52).

Tissue and Cellular Heterogeneity May Drive Heterogeneity in the Population with Obesity

We have addressed several issues such as sex, race, ethnicity, age at onset of obesity, and genetics as they relate to heterogeneity at the population level. However, heterogeneity of obesity is often ignored at the tissue and cellular level in pre-clinical- and population-level studies. Here we use adipose tissue as a case in point (53, 54). Adipose tissue presents several levels of heterogeneity with distinct properties and functions, especially in white adipose tissue (WAT) (53), as illustrated by 1) different types of adipose tissues known as brown, white, and beige adipose tissues, with different locations within the body, and 2) cellular heterogeneity in cell types and cell size, as adipose tissue is composed of several cell types, including pre-adipocytes, stem cells, immune cells, and adipocytes among other cells.

WAT is a primary storage organ of triglycerides during energy excess. WAT influences systemic metabolism not only through availability of these stores that can be released as fatty acids when needed, but also through secretion of numerous hormones and adipokines secreted by adipose tissue (55). In contrast, brown adipose tissue (BAT) is a major driver of thermogenesis and energy expenditure through a specialized mitochondrial protein—uncoupling protein 1 (UCP-1)—and heat generation in both humans and rodents (56, 57). Over the past decade, beige adipose tissue emerged as a third type of adipose tissue. Beige adipocytes are brown like adipocytes and positive for UCP-1 and arise within white fat (also named brite for “brown in white”). Published research shows that these tissues not only differ in metabolic functions, but they also exhibit distinct molecular differences (58).

Adipose tissue expands through hyperplasia (proliferation then differentiation of adipose stem cells or pre-adipocytes) and/or through hypertrophy (increased mature adipocyte cell size). The latter, especially for WAT, has significant implications on obesity-related diseases. Indeed, the inability of adipocytes to expand and continue storing energy leads in part to “spillover” of lipids into non-adipose tissues such as liver, muscle, and pancreas, causing lipotoxicity and associated metabolic dysfunctions, including insulin resistance (59, 60). Moreover, adipocytes come in different sizes (61), and it is generally recognized that smaller adipocytes are associated with insulin-sensitive phenotypes while large adipocytes are associated with insulin resistance. This has been well illustrated in several animal models—specifically, insulin receptor knockout models as well as angiotensinogen and angiotensin II receptor transgenic/knockout models (62, 63).

It is important to recognize that differing cell types, notably adipocytes, found in various adipose tissue depots

are developmentally distinct. Thus, differences in fat distribution across depots are driven, in part, by the ability of each unique progenitor cell to grow and differentiate. This developmental programming is under the control of a unique transcriptome, which is likely further regulated by sex hormones (58). Furthermore, there is a strong appreciation for heterogeneity in numerous immune cell populations, each with their own specialized metabolic profile, within differing WAT depots that control the inflammatory tone (64).

Understanding the aforementioned heterogeneity of adipose tissues and adipose tissue distribution, in addition to the heterogeneity of adipocytes and immune cells, using mouse models and well-defined human fat samples may help stratify the different types of “obesities,” which will ultimately result in designing better targeted interventions for metabolic diseases. Specifically, given the limited amount of BAT in humans, a promising target is WAT, including understanding mechanisms and interventions that can reduce it and/or increase potential conversion of white into brite adipocytes. Moreover, development of animal models that better mirror heterogeneity in metabolism and distribution of adipose tissues may shed light on how adipose tissues impact whole body metabolism and lead to different metabolic outcomes of obesity.

Collaborative Cross and Diversity Outbred Mouse Populations Are Powerful for Modeling Complex Diseases and May Increase Understanding of the Heterogeneity of Obesity

Diverse populations are key to understanding heterogeneity in obesity. Our major breakthrough in understanding the genetic underpinnings of obesity’s complexity have come from large, population-based genetic consortia (65–75). Yet even among these large studies, there is generally a historic gap in studies in multiethnic human populations, with most work in European Americans (76–78). This is a problem because of poor transferability of European-American trait-associated variants to multiethnic populations (79, 80). Of course, new data are starting to emerge from multiethnic populations, which are critical for investigating the heterogeneity of obesity. As one example, a genome-wide association study (GWAS) from 100,418 adults from the multiethnic Genetic Epidemiology Research on Adult Health and Aging (GERA) cohort identified 30 novel BMI autosomal loci (81). For instance, *KDM4C* was identified, which is a transcription factor involved in regulating adipogenesis (81). Some of these data are publicly available and are a rich resource for further investigation (82).

Most animal studies have capitalized upon standardized genomic backgrounds that are homogeneous (homozygous inbred). Further, animal studies have typically only interrogated variation at a single locus. Thus, these types of studies provide a poor model for human variation relative to complex multigenic diseases, like obesity. In contrast, novel heterogeneous mouse populations, like the collaborative cross (CC) and diversity outbred (DO), better approximate

human populations in terms of genetic diversity and thus are excellent for investigating complex disease biology (83).

Homozygous inbred lines of mice have been a useful pre-clinical experimental research model with success in basic and biomedical research with qualifications (84). To aid quantitative research similar to human GWAS, new mouse models and approaches have been created and used for genetic analysis (85–90). Hybrid mouse diversity panels, the collaborative cross recombinant inbred lines (CCRIL) and DO mice, are representative of haplotype association mapping and linkage analysis approaches in pre-clinical experimental population-based mouse models. Hybrid mouse diversity panels are a powerful tool with more than 8 million single nucleotide polymorphisms (SNPs) but are limited by significant intervals that are identical by descent (IBD) in some or all laboratory-derived homozygous inbred lines (91, 92). This approach may be limited for robust quantitative trait loci (QTL) analysis for some phenotypes.

The CCRIL and the DO mice have different strengths and potential limitations. The CCRIL mice were created through a multiple advanced generation intercross using 8 homozygous inbred lines of mice selected based on particular attributes (93). The DO mice were created from early progenitors of the established CCRIL in 2 steps starting from a founding population of 150 sister–brother pairs of partially inbred CC lines sampled at the sixth filial generation from the second generation (G2F6) (94). Simulated rounds of mating in which females and males were paired at random with the constraint that sib-mating was prohibited were used (94). The final inbred lines of the CCRIL and the randomized DO mating project contain approximately 45 million single nucleotide polymorphic and structural variants and a 12% minor allele frequency (MAF). Together, they represent powerful tools for quantitative genetics and identifying QTL based on the variance contributed by one or more founder haplotype of origin (95, 96). The 8 founder lines contributed significant genetic diversity and a high MAF for genetics to aid quantitative trait analysis to identify genes, and bioinformatic analysis to explain significant variance associated with phenotype. These discovery tools further enable reverse genetics studies to demonstrate causal relationships using the founder lines or the CCRIL. The genetic sequence and identification of phenotype-associated QTL identified candidate gene SNP or structural variants and tools to aid mouse to human translation are available on the Mouse Genome Informatics database at The Jackson Laboratories (97, 98). For further information, please refer to The Jackson Laboratory Mouse Genome Informatics (MGI; <http://www.informatics.jax.org>) and Mouse Phenome Database (MPD; <https://phenome.jax.org/>).

It is important to note that the creation of loss and/or gain of function mutants based on “single locus” of origin (homozygous inbred strain) used in genetically altered mouse models for pre-clinical research does not apply to the CCRIL and DO mice models. Each CCRIL or DO mouse has 8 different alleles (haplotype) at each genetic locus.

Each CCRIL is isogenic (>95% homozygosity at each locus) allowing for co-isogenic controls in experimental balanced block design.

Many homozygous inbred strains derived in the laboratory, principally from the *Mus musculus domesticus* subspecies, have significant IBD. IBD limits genetic diversity at specific loci and significantly decreases the statistical ability to identify phenotype-specific protocol-driven QTLs based on non-synonymous SNPs and/or copy number variations. Thus, our understanding and accounting for the multigenic basis of the phenotypic trait to explain phenotype variance can be decreased. Depending upon study design, the randomly bred genetically diverse DO mice and the isogenic inbred CCRILs capture a similar magnitude of genetic diversity. Together, they provide increased opportunities to develop methods necessary to extrapolate between these genetically diverse mouse models and humans based on similar phenotype and genotype.

Weight gain and loss risk variants identified in DO mice studies can be tested in selected CCRIL lines mice with CRISPR/Cas9 modifications to investigate specific allelic variants and their mechanisms to demonstrate proof of causality. For example, population-based genetically diverse mouse models can be used to dissect complex traits related to nutrient overload (83, 99). Several studies in CCRIL and DO mice have demonstrated the use of these population-based models to identify QTLs for explaining human phenotypic variation (100–103).

Taken together, we consider the randomized DO mice as forward genetics or discovery models and the isogenic CCRILs for corroborating phenotypes and QTLs leading to hypothesis-based research with isogenic controls as well as traditional comparisons of phenotypic responder and non-responder F1 and F2 outcrosses. For instance, DO mice are being used as a discovery model to determine why some obese mice have improved hyperinsulinemia and hyperglycemia with select dietary interventions whereas other mice have impaired hyperinsulinemia and hyperglycemia (99). To further exemplify, in our DO mice studies focused on the heterogeneity of obesity based on operational paradigms, we are using series of DO mice cohorts (50 to 100 of each sex) over time to gain power for linkage analysis and discovery of candidates that explain the haplotype(s) of origin and the majority of the operationally defined phenotypic variance (research paradigm dependent). Once the haplotype is identified that explains the phenotypic variance, we can use the CCRIL with and without the genetic locus specific haplotype and retesting, and compare and contrast outcomes (JE French et al., manuscript in preparation). This also allows for use of the appropriate CCRIL for creating haplotype-specific controls and experimental groups to test gene × diet interactions. We are working toward using experimental clinical designs and phenotypic/genetic analysis outcomes and incorporating the elements of those designs based on the features of the CCRIL and DO mouse models to test complementarity between SNP-based GWAS or family linkage analysis approaches.

Integration of -Omics and Computational Approaches to Tackle the Heterogeneity of Obesity

For many decades, obesity research has addressed mechanisms involving 1 single factor (e.g., using a single type of data such as either genetics or metabolomics rather than both genetics and metabolomics) in a single model (e.g., human or single inbred mouse strain) at a single point in time. Yet current advances in computational efficiency, data science, and measurement technologies provide outstanding opportunities for investigating the heterogeneity of obesity. For example, we can now start to integrate a wide range of -omics (i.e., metabolomics, lipidomics, proteomics, genomics, microbiome) into pre-clinical and clinical studies to ultimately establish the underlying mechanisms and potential treatment approaches across different subtypes of obesity. Although this approach has been rarely applied to obesity heterogeneity, there are emerging studies that integrate various -omics analyses in the human population. To exemplify, a recent epidemiological study examined associations between the gut microbiota and the plasma metabolome with blood pressure in a Chinese cohort. The data revealed unique microbiota and metabolite signatures (notably of acyl-carnitines and differing lipids) that were associated with systolic and diastolic blood pressure (104). Additionally, using complex multi-omics data in combination with integrative analysis and systems biology along with expertise in obesity and metabolism has the potential to transform current understanding of the heterogeneity of obesity.

The rise of publicly available structured biomedical knowledge has allowed the creation of large-scale knowledge integration projects such as the NCATS Biomedical Data Translator (<https://doi.org/10.1111/cts.12591>), including the creation of the ROBOKOP Knowledge Graph (KG) (<https://doi.org/10.1021/acs.jcim.9b00683>). As an example, the ROBOKOP KG compiles information from a dozen public sources and contains information on over 4 million biomedical entities including genes, diseases, phenotypes, chemicals, anatomical features, and sequence variants as well as over 12 million relationships between these entities. The KG, which is available for browsing and download at <http://robokopkg.renci.org>, serves as an integration point for observed obesity associations. These associations are loaded into the graph as new relationships, such that subsequent database queries return association data combined with background information, providing the framework for a mechanistic interpretation of new associations focused on the heterogeneity of obesity.

The flexibility of knowledge graphs to integrate heterogeneous data also allows a systematic representation of information across species. For instance, the Monarch Initiative Knowledge Graph relates phenotypes, anatomical features, and genes across model organisms so that genotype/phenotype relations observed in, for instance, mice, can be used to suggest or support orthologous relations in humans (105). The structure of the KG, therefore, allows for

parallel mechanisms to be explored across species without losing context of the particular organism.

Risk Factor and Genetic Clustering May Reveal Subtypes of Obesity

An additional approach for tackling the identification of differing types of “obesities” will be to cluster differing biomarkers of disease, risk factors, or even genetic pathways. These biomarkers could be well established clinical measures or could even be the identification of new and validated parameters from -omic approaches described earlier. Here we describe a couple of examples from studies focused on the heterogeneity of diabetes.

As an example, a study used k-mean and hierarchical clustering analysis of nearly 9000 newly diagnosed participants with diabetes. The clusters were defined by BMI, HbA1c, age at diagnosis of diabetes, circulating concentration of glutamate decarboxylase antibodies, and homeostatic model assessment of pancreatic β -cell function and insulin resistance. These parameters were then associated with patient record data, including complications of diabetes and use of prescription drugs. The analyses revealed 5 groups of patients with diabetes, with each cluster having unique characteristics and risks. For instance, one notable finding was that those that were the most insulin resistant had the highest risk for fatty liver and kidney disease (106, 107).

To further exemplify, in another study, GWAS results were clustered using Bayesian non-negative matrix factorization for 94 type 2 diabetic genetic variants and 47 type 2 diabetes traits (108). Analyses from this study revealed 5 genetic clusters with distinct traits that appeared to represent unique mechanistic pathways that drive the onset and/or progression of type 2 diabetes. This approach highlighted the possibility of stratifying individuals based on distinct genetic pathways that could predict physiological outcomes. Taken together, these results underscore the potential utility of these approaches that can be applied to the field of obesity to drive future precision medicine and precision nutrition studies. In addition, establishing underlying cellular and molecular mechanisms with rodent models will aid in our understanding of why some pathways favor specific physiological outcomes.

Best Practices for Animal and Human Research on the Heterogeneity of Obesity

There is a strong need for establishing uniform practices in the study of obesity heterogeneity using pre-clinical and clinical models to ensure rigor and reproducibility. There are several variables that we discuss below that are often ignored in the field. One major variable is the lack of consideration for gene \times diet interactions. Obesity and its complications such as type 2 diabetes and CVDs are influenced by multiple genetic and environmental factors, including diet behaviors. Genome-wide association studies are the best means of confirming known and discovering novel variants associated with obesity. Some studies have been successful in identifying genetic variants and their interactions with environmental

factors in influencing the disease process and/or risk factors (109–111). However, most of these studies are lagging behind with respect to lifestyle interactions (e.g., sleep, physical activity, stress, smoking, and alcohol consumption), despite known recognition that inclusion of lifestyle intake data reduces the noise in genetic signals (112–114). Studies that have reported and replicated gene–nutrient interactions affecting obesity have mostly focused on macronutrients, mainly fat and carbohydrate intake (115–117), with few focusing on meal patterns. Key genes whose interactions with nutrients/meal patterns have been reported and replicated across studies include apolipoprotein A2 (*APOA2*), fat mass and obesity associated (*FTO*), melanocortin 4 receptor (*MC4R*), lipoprotein lipase (*LPL*), and peroxisome proliferator activated receptor gamma (*PPAR γ*) (118–122).

Diet can regulate gene expression by affecting transcription (RNA processing and stability), RNA translation, and proteins and metabolite processing. In turn, metabolism of nutrients is affected by the genetic sequence and architecture of the individual (123, 124). Evolutionarily, nutritional environments seem to be the major determinants of human variation, given that populations vary in requirement for foods and response to diet (124). Thus, the fields of nutrition and genetics are intertwined; studies of human or animal genetics are not complete without taking into consideration nutritional variability in the population with obesity. However, diet \times nutrient interactions account for a small portion of the variation in obesity. Another component of lifestyle that has evoked interest is the role of gene by physical activity interactions in obesity. Genes such as angiotensin-converting enzyme (*ACE*), angiotensinogen (*AGT*), alpha actinin 3 (*ACTN3*), and *FTO* have been consistently shown to interact with physical activity and to be associated with adiposity/obesity (125–128). Other studies have reported interactions of genetic variants with other factors such as sleep (129, 130), stress (131), smoking (132, 133), alcohol intake (134), and even socioeconomic status (135–137).

It is important to note that gene by lifestyle interactions in obesity have been mainly examined using genome-wide and candidate gene association studies. Several models have been proposed to estimate and analyze interaction effects with obesity, where most are regression based. Some of the issues that need to be taken into consideration while examining gene by lifestyle interaction effect on obesity include selection of genetic models (additive vs. dominant vs. recessive), interaction models (additive or multiplicative terms), and type of confounders and time of exposure (113, 138).

Another limitation is the differences in the instruments used to measure dietary intake. Dietary intake assessment is usually conducted using food records, 24-h recalls, and FFQs. The ability of food records and 24-h recalls to capture usual intake depends on the number of days assessed. Similarly, the accuracy of FFQs, designed to capture long-term intake patterns, is also affected by the number of days assessed along with the number and relevance of foods included, in addition to the recall bias. All these factors need to be

considered and standardized across studies to ensure rigor and reproducibility. One approach that can be integrated into studies is the use of mobile technology and wearable-based detection approaches, which of course have their own challenges (139–141).

Another constraint in advancing the science of obesity heterogeneity is the lack of uniformity in diet composition to achieve an obese phenotype. For example, the fat source for high-fat diets varies routinely in pre-clinical experiments. A range of fat sources are implemented in the experimental diets, including coconut oil, palm oil, lard, milkfat, and mixed oils (142). Similarly, a control diet for high-fat feeding studies is often a purified mouse diet or at times, erroneously, a standard mouse chow (142). However, the ingredients found in a standard mouse chow are highly variable, containing many additional substances such as pesticides, heavy metals, and phytoestrogens (143). It is no wonder that reproducibility of effects or lack of translation from animal to human models is often problematic. Greater attention must be paid to diet in design and interpretation of studies, particularly relevant to consistency in controls across laboratories, to make rigorous conclusions about any given macro- or micronutrient. Like the use of the ARRIVE (Animal Research: Reporting of In Vivo Experiments) checklist and other guidelines for mouse studies (144, 145), we are in need of strong nutrition guidelines for obesity research.

The case in point about control diets also applies to human intervention studies. For instance, what are appropriate controls for human dietary supplement studies? If we stick with studies of dietary fat as a variable, then what are appropriate placebo controls?

Again, a limiting factor is the lack of collection of high-quality diet data in population, cohort, and clinical studies. The few studies that incorporate diet measures rely upon a wide range of methods used to capture dietary intake, which makes cross-study harmonization very difficult. Given the role of diet/nutrients in obesity, there is a need to integrate high-quality nutritional assessment tools and nutritional biomarkers in population, cohort, and clinical studies, and to use appropriate experimental models to attain proper translation to human conditions.

Understanding obesity as a heterogeneous disease with complex mechanisms and etiologies can take us further to unique prevention and treatment strategies tailored to the individual. In this paper, we have provided some fundamental gaps in knowledge of the heterogeneity of obesity ranging from the cellular level (e.g., heterogeneity of adipocytes and immune cells across fat depots) to population perspectives (age at onset of obesity, sex, host genetics). It was not our intent to address the full scope of individual predisposition factors and the full range of environment and lifestyle factors. We specifically focus on a set of factors important to translational science in synthesizing human and animal models of obesity heterogeneity. As such, we are not including a wide range of other factors that are important but may relate only to human or only to mouse studies. Taken together, we propose the following measures to improve

studies of obesity heterogeneity and to enhance translation from mouse to human:

- 1) Build translational teams to address heterogeneity of obesity. Integrating expertise across basic, computational, clinical, and population for true translational science and integrative analyses is critical to making headway in understanding the heterogeneity of obesity. Application of -omic (i.e., metabolomics, lipidomics, proteomics, genomics, microbiome) and computational approaches will also be key.
- 2) Analyses of validated SNPs that could account for differences in nutrient metabolism. This will take us one step closer to addressing the neglect of gene \times diet interactions. Furthermore, increased utilization of population-based models such as DO and CC mice as models for complex diseases, will provide key information about novel SNPs in the heterogeneous population with obesity.
- 3) Experimental diets for rodent and human studies should undergo rigorous quality control analyses by investigators prior to and during the course of a study and the results should be reported.
- 4) The bulk of current studies that rely on 45% and 60% kcal from fat diets do not model human intake of carbohydrates and fats. Investigators at the pre-clinical level should start the use of newly emerging obesogenic diets that model human macronutrient intake (146).
- 5) Account for sex differences in human and mouse studies. While there is increasing appreciation for sex differences, this area of research needs to be further expanded as sex differences will contribute toward differences in various measured outcomes. Notably, rodent studies are still heavily focused on male mice although there is a greater appreciation for conducting experiments with females based on a recent push from the NIH. These studies will need to address sex differences from the population level to differences in underlying mechanisms of action.
- 6) Account for the age at onset and duration of obesity. This is particularly relevant when comparing results between mice and humans.
- 7) Incorporate race/ethnic diversity in human studies as an important source of heterogeneity and a marker for structural factors that contribute to disease.

Conclusion

Collectively, there is a critical need to understand the underlying sources of heterogeneity in the population with obesity. Translational studies spanning mouse and human populations offer one such direction of research. Sources of heterogeneity range from sex/race difference, age at onset of obesity, differing genetic backgrounds, diversity in the diet, variations across individuals in their -omic profiles (microbiome, metabolome, lipidome, genome, proteome), and differences among individuals in their underlying cellular profiles within key tissue depots such as brown and

white adipose tissue. Addressing the underlying causes of heterogeneity in obesity and developing precision medicine and precision nutrition treatment approaches will rely on large-scale integration of the next generation of population-based mouse models with human clinical studies. Finally, there is a need to develop better practices that will allow for strong rigor and reproducibility in the study of obesity heterogeneity.

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